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Effect of grazing and season on the chemical composition of Mediterranean shrub species in Doñana Natural Park, Spain

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ABSTRACT

We evaluated the interaction between seasonal changes and grazing pressure on the chemical composition of six Mediterranean shrub species (*Cistus salvifolius*, *Halimium halimifolium*, *Myrtus communis*, *Phillyrea angustifolia*, *Pistacia lentiscus* and *Rosmarinus officinalis*). In each season, condensed tannins, total phenols, and N and C concentrations were determined by NIRS spectroscopy. In the case of the nitrogen, tannin and phenol contents, all species presented a significant interaction between season and grazing. Carbon content did not differ significantly between grazed and ungrazed areas, but there were significant changes in carbon content over season. Nitrogen content was higher during vegetative growth periods, but fell dramatically in summer. Species-specific responses of the analyzed compounds were detected: compared to the other species, concentrations of tannins and phenols were lower in grazed *H. halimifolium* and *M. communis* individuals. Multiple regression models revealed a significant relationship between consumption of species and their carbon and tannin contents (consumption was higher when species present decreased carbon and tannin contents). This study suggests that, contrary to popular belief, goats are selective grazers; goat grazing is not random. During grazing, goats increase their nutritional intake (N content) while minimizing consumption of secondary compounds (tannins and phenols).

1. Introduction

Wild or domestic livestock grazing has been described as an important biological factor that determines the composition and structure of Mediterranean ecosystems (Carrion et al., 2003). Goats are polyphagous animals that feed on a great variety of species; however, they prefer certain species while rejecting others (Mancilla-Leytón et al., 2012; Papachristou et al., 2007). Such selective behavior may be governed by a series of factors: plant abundance, plant chemical characteristics (nutrients, secondary compounds, etc.) and vegetation heterogeneity (Baraza et al., 2009; Papachristou et al., 2007). According to Merrill and Taylor (1981), diet selection in goats is primarily determined by the variety and relative abundance of each available plant species. Depending on the nutritional quality or concentration of chemical defenses in the plants, selection by goats can also take place between different plant species or different individuals of the same plant species (Barroso et al., 2001; Provenza et al., 2003; Riddle et al., 1996). Nutritional quality can therefore differ between different species, or between individuals or branches of the same species (Orians and Jones, 2001). These variations, which can be measured as differences in nutritional value as well as in the morphological characteristics of twigs and tissues (Hartley and Jones, 1997), increase or diminish herbivorous consumption among different species, plants of the same species, or even parts of the same plant (Alonso-Díaz et al., 2008; Baraza et al., 2009; Riddle et al., 1996).

As previously stated, chemical composition (as secondary compounds) is an important factor in the consumption of a species. Anti-herbivory defense system chemicals (secondary compounds) may be a constitutive defense (always present in the plant, as in the aromatic species, Alonso-Díaz et al., 2008; Distel and Provenza, 1991) or may be induced as a response to grazing (Baraza et al., 2009; Barroso et al., 1995). Thus, the diet of herbivores depends not only on plant availability but also on the presence of these secondary compounds; almost all shrub species contain potentially toxic or digestibility-reducing secondary compounds (Bryant et al., 1991), such as phenols and tannins, which strongly influence food selection by herbivores (some species are positively selected, while others are partially or totally rejected) (Alonso-Díaz et al., 2008;

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Dziba et al., 2003; Ngwa et al., 2000). Detection of these changes in secondary compounds over time contributes to the understanding of how livestock select their diet, and the factors that influence this diet selection. Such knowledge would be of considerable value for the development of rational techniques leading to appropriate management of the shrublands they occupy.

The objectives of this study were i) to explore the temporal evolution (seasonal changes) of the chemical composition of six Mediterranean shrub species subjected to long-term grazing by large domestic herbivores, ii) to relate variation in browsing intensity (measured by Mancilla-Leytón et al., 2012, Table 1) and chemical composition in terms of condensed tannins, total phenols, and concentrations of nitrogen and carbon. We assumed that changes in leaf biochemical composition were related to both phenology and grazing.

2. Material and methods

2.1. Data sets

Samples were collected from the understory in a 100 ha pine forest (*Pinus pinea*) located in Doñana Natural Park (37°14′N, 6°20′W, SW Spain). The climate of the study site is Mediterranean, with a mild wet winter (monthly average temperature is 10 °C in December and January) and a long dry summer (mean temperature of 25 °C in July and August). Mean annual rainfall is around 540 mm, with 80% of this precipitation occurring between October and March (Mancilla-Leytón et al., 2013). The geological substratum is quarcitic sand, with an undulated relief. Soils are shallow, acidic and poor in nutrients (<5% organic matter) (Merino et al., 1990).

The study area is used for timber production, hunting (rabbit, partridge) and grazing of wild and domestic animals. Wild herbivores (deer) were excluded in 1970 and domestic goats in 2002, the study area remained ungrazed for a period of five years prior to the reintroduction of domestic goats (2007). During this five-year period of livestock exclusion, the natural vegetation was not subject to any form of management and consequently underwent rapid growth and expansion. In 2007, before reintroducing the goats, nine 0.25 ha fenced exclusion plots were established in the study area. In spring 2007, a herd of 350 adult female Payoya goats (average weight of 45 ± 5.3 kg, age 2.9 ± 1.4 years) was introduced into the study area at a stocking rate of 2.7 goats ha^{-1} yr^{-1} (characterized as moderate grazing). The goats browsed the entire area for three consecutive days, before spending the fourth day outside the area (280 days grazing). This livestock management may be considered to be semi-extensive, although in order to graze the 100 ha area in a uniform manner, the goats were closely controlled and moved around by a shepherd (Mancilla-Leytón et al., 2012).

The plant species used in the study were six common Mediterranean shrub species: *Cistus salvifolius* L. and *Halimium halimifolium* (Cistaceae), *Myrtus communis* (Mirtaceae), *Pistacia lentiscus* (Anacardiaceae) *Phillyrea angustifolia* (Oleaceae) and *Rosmarinus officinalis* (Lamiaceae). The species selected were among the most abundant and evenly distributed in the study area (semideciduous, resprouting and aromatic species; representing 80% of the understory cover) and were therefore subject to extensive grazing by the goats (Mancilla-Leytón et al., 2012).

Table 1
Goat consumption (%) obtained by direct observation and cover (%) of shrub species found in the understory of a Mediterranean pine forest under goat grazing in Doñana Natural Park (SW Spain), from spring 2011 to summer 2012.

Species	Autumn	Winter	Spring	Summer	Cover
Cistus salvifolius	18.7	27.5	15.9	5.0	13.3
Halimium halimifolium	25.2	11.6	10.4	5.7	9.4
Myrtus communis	30.1	27.7	36.4	67.6	9.1
Phillyrea angustifolia	0.6	0.1	2.1	0.7	1.2
Pistacia lentiscus	7.3	1.9	4.2	4.1	6.6
Rosmarinus officinalis	0.0	7.9	2.3	0.0	14.0

Values from Mancilla-Leytón et al. (2012).

Current year foliage samples from each plant species were collected once per season (spring 2011, summer 2011, autumn 2011 and winter 2012). Samples of terminal twigs with their leaves were cut (in a quantity similar to that of a bite) from 45 different individual plants of each species and each area (9 groups of 5 individual plants inside [ungrazed area] and 9 groups outside [grazed area] the exclusion plots). Plants of similar characteristics were randomly chosen and marked in order to facilitate collection of data from the same individuals at every sampling session. From each plant, 15–20 terminal twigs were randomly cut at midday, transported to the laboratory and weighed; they were then oven-dried at 60 °C for 48 h. Measurements from the 5 individuals were averaged, thereby producing paired sampling within each of the sites (grazed and ungrazed areas), each of which were considered as individual experimental units.

2.2. Near infrared reflectance spectroscopy (NIRS) analysis

NIRS represents a very useful tool in terms of large scale monitoring since it is a fast, cost-effective and non-destructive technique that can provide information regarding animal diet and nutrition (Foley et al., 1998). Contrary to wet chemical analysis, this technique features small sample sizes and short analysis times while incurring lower costs (from 80% savings in lab costs, according to Aragones (1997)).

A total of 2160 dried samples were ground in a cyclone mill, passed through a 1 mm mesh and scanned using a NIR spectrophotometer (NIRSystems 6500, Silver Spring, MD, USA) in Centre d'Écologie Fonctionnelle et Évolutive (CNRS Montpellier, France). For each measurement, 32 scans were taken at 2 nm intervals over a range from 400 to 2500 nm in order to produce a mean spectrum with 1050 data points. Using ceramic standards, the internal software evaluated the spectrum of apparent reflectance (R). The spectral data recorded were then processed and stored in absorbance units (A) equal to log ($1/R$). Data analysis was conducted using the ISI software system (Shenk and Westerhaus, 1991).

2.3. Chemical composition

Following NIRS analysis, 70 representative samples were selected for analysis in the laboratory. We applied the SELECT algorithm, using WinISI™ III v.1.60 (Infrasoft International, LLC), to extract the 70 most representative samples in our spectral population, which included all the seasons, species and grazing. Total carbon and nitrogen concentrations were measured using a flash elemental analyzer (EA1112 Series; Thermo Finnigan, Milan, Italy). Condensed tannin concentration was measured spectrophotometrically using the butanol–HCl method (Coulis et al., 2009). Total phenols were analyzed according to Coq et al. (2010). The mean values were used for NIRS calibration and validation.

2.4. Population structuring

All sample sets were examined using population-structuring software in order to identify spectral outliers. Principal component analysis (PCA) was used to identify patterns in the group of spectra that make the greatest contribution to variation among the spectra. An average Mahalanobis distance (Global H) was calculated and H values for individual samples were standardized by dividing